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Antimicrobial and antifungal agents derived from clay minerals

Part IV *Properties of montmorillonite supported by silver chelate of hypoxanthine*

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Montmorillonite supported by Ag^+–chelate of hypoxanthine was synthesized and heat-treated at 200 °C intervals below 800 °C under nitrogen. These samples were subjected to examinations of the structure and properties including antimicrobial activity. Hypoxanthine itself did not possess antimicrobial activity to both *Staphylococcus aureus* and *Escherichia coli*, but montmorillonites supported by the Ag^+–chelate exhibited activity. Hypoxanthine in montmorillonite decomposed at 400–600 °C; and Ag metal particles deposited after heating to 600 °C and grew with raising heating temperature. The Ag^+–chelate montmorillonite showed clear antimicrobial activity even after heating to 800 °C.

1. Introduction

Montmorillonite supported by Ag^+ (Ag–Mont) has a strong antimicrobial activity [1]. However, when its aqueous sol is allowed to stand, such Ag^+ was reduced easily into Ag metal particles, leading to lowering of antimicrobial activity and to changing of its colour from ochre to black. In the previous work [2], this reduction was prevented by incorporating Ag^+–chelates of 1,10-phenanthroline and 2,2′-bipyridine instead of Ag^+ itself. However, two disadvantages became clear: (i) both chelating agents were toxic materials; (ii) it was difficult to make clear what the antimicrobial activity results from, because both chelating agents have activity as well. In the present work, therefore, hypoxanthine without antimicrobial activity was used to prepare the chelate with Ag^+ by considering (i) and (ii), namely, the montmorillonite with Ag^+–hypoxanthine chelate was synthesized and its structure and properties including antimicrobial activity were examined.

2. Experimental procedure

2.1. Materials and preparation procedure

Montmorillonite used has been reported elsewhere in detail [1, 3]. $AgNO_3$ and hypoxanthine ($C_5H_4N_4O$) are the chemical grade.

About 3 wt % montmorillonite aqueous sol with a known cation exchange capacity (CEC) was prepared. Hypoxanthine of double the molar quantity of the CEC was dissolved in hot water. $AgNO_3$ was dissolved in deionized water by an equal molar quantity of the CEC. Both solutions were mixed to form Ag^+–chelate sol to which the montmorillonite sol was added, followed by stirring at 80 °C for 2 days. The resulting montmorillonite supported by Ag^+–hypoxanthine chelate (HAM) sedimented rapidly rather than montmorillonite, indicating incorporation of the chelate. The white colour of the aqueous sol of HAM did not change after standing for about one month. After washing with deionized water, the sol was concentrated centrifugally and subjected to freeze-drying.

HAM was heated below 800 °C in a stream of N_2. The heating rate and the residence time at a prefixed temperature were 5 °C min^{-1} and 1 h, respectively. A sample is labelled by appending a heating temperature (°C) after HAM, e.g., HAM–0 and HAM–600 are HAM before and after heating to 600 °C. Montmorillonite (Mont) as a reference was also heated in the same way

2.2. Measurements

Powder X-ray diffraction was carried out by using the Ni-filtered CuK_α radiation. IR spectra were taken by the KBr tablet method. Thermogravimetric analysis (TGA) was carried out with a heating rate of 5 °C min^{-1} up to 1000 °C in a stream of N_2. Carbon content was measured by a combustion method using tin particles as a combustion accelerator. Silver content was measured by using inductively coupled plasma (ICP) emission spectroscopy. Transmission electron microscope (TEM) was used to observe Ag particles in a specimen. Antimicrobial activities

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against *Staphylococcus aureus* ATCC 6538p and *Escherichia coli* IFO 3301 were evaluated by the so-called halo method. These measurement methods have been reported elsewhere in detail [1, 2].

3. Results

3.1. X-ray diffraction

Changes of X-ray diffraction profiles of montmorillonite with heating have been reported elsewhere [3]. Figs 1 and 2 show X-ray diffraction profiles and the basal spacing (d_{001}) of HAM before and after heating. The d_{001} of HAM before heating was 1.22 nm which was larger than that of montmorillonite by 0.29 nm, indicating incorporation of Ag^+–hypoxanthine chelate. This value scarcely changed up to 400 °C, but decreased to 0.98 nm at 800 °C. This value roughly corresponds to d_{001} of montmorillonite. Montmorillonite is destroyed after heating to 800 °C [3] but HAM gave weak diffraction peaks resulting from the layered structure after heating to 800 °C. Peaks of silver metal appeared at 600 °C and strengthened at 800 °C.

3.2. Ag and C contents

Fig. 3 shows changes of Ag and C content of HAM with heating temperature. HAM before heating showed 9.5 wt % of Ag content. This value means that almost all of the CEC was exchanged by the

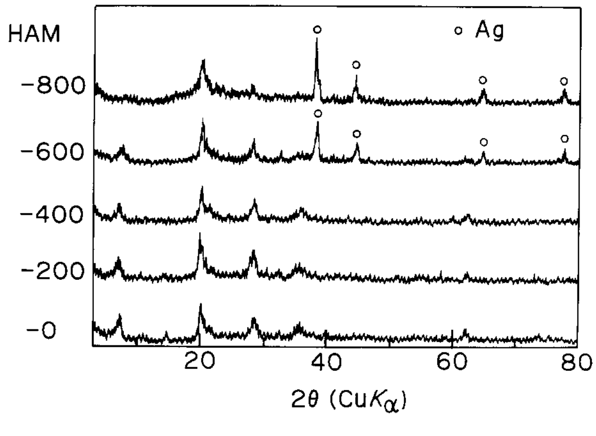

Figure 1 X-ray diffraction profiles of HAM before and after heating.

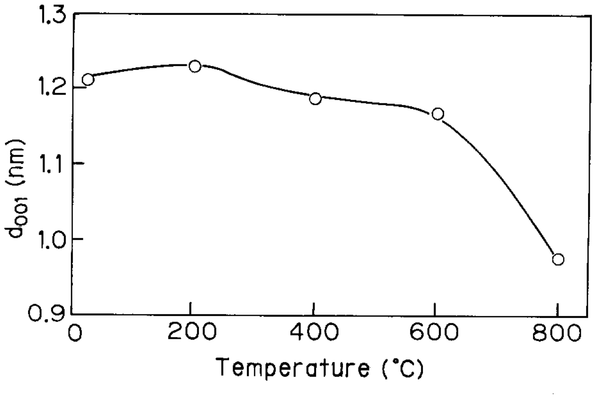

Figure 2 Change of d_{001} of HAM with heating temperature.

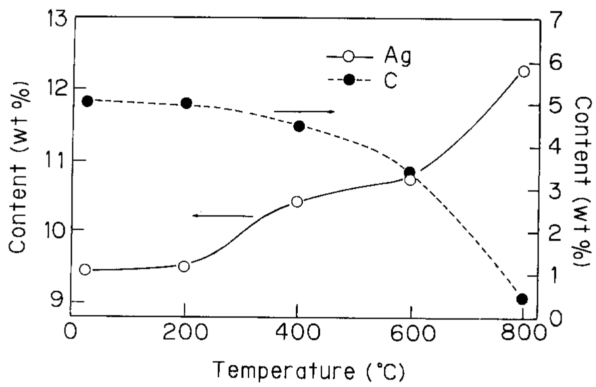

Figure 3 Changes of Ag and C content in HAM with heating temperature.

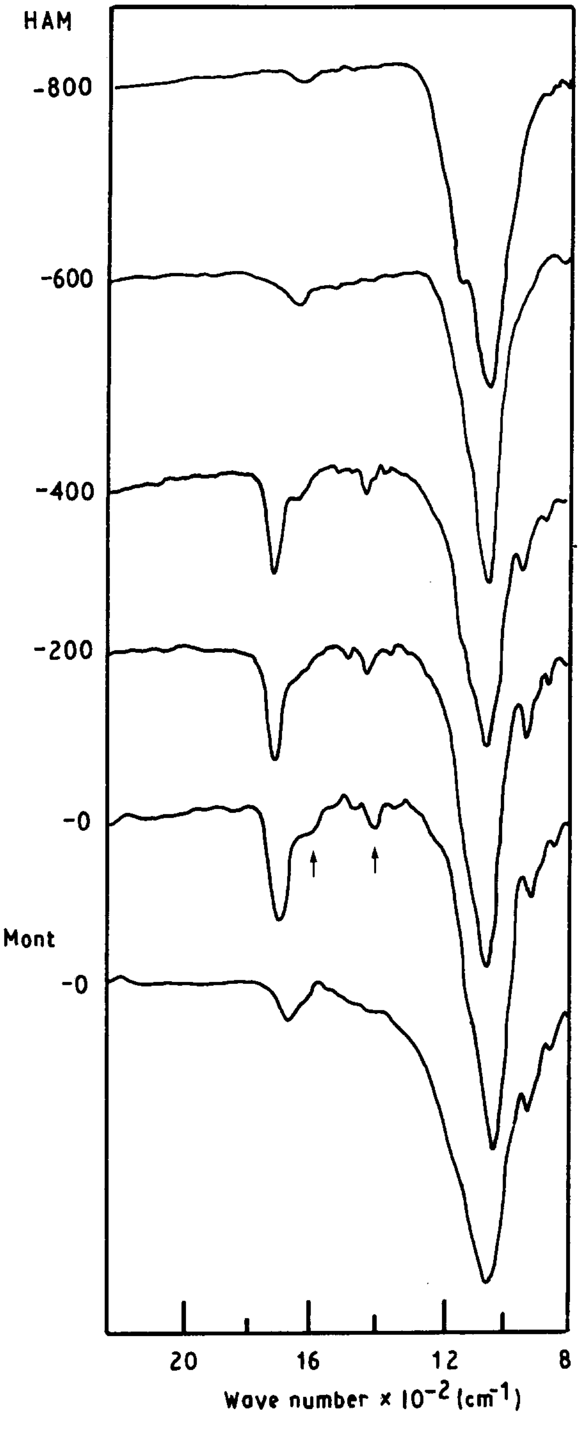

Figure 4 IR spectra of HAM before and after heating.

Ag$^+$–chelate. Ag content was not changed up to 200 °C. Relatively large increases of Ag content occurred at 200–400 °C and 600–800 °C.

C content of HAM before heating was 5.1 wt % which was scarcely changed up to 400 °C. At higher temperatures, C content decreased considerably to reach to 0.5 wt % at 800 °C. On the basis of Ag and C contents of HAM before heating, the coordination number of hypoxanthine to Ag$^+$ was nearly unity.

3.3. IR spectra

Fig. 4 shows IR spectra of raw montmorillonite and HAM before and after heating. HAM–0 showed absorptions at 1400 cm^{-1} assigned to C=N stretching and 1600 cm^{-1} assigned to C=C, C=N stretching of hypoxanthine. Such absorptions scarcely changed in HAM–200 and HAM–400. However, HAM–600 did not show these absorptions, indicating the decomposition of hypoxanthine.

TABLE I Antimicrobial tests

Sample	*Staphylococcus aureus*	*Escherichia coli*
Hypoxanthine	−	−
HAM–0	+	+
HAM–200	+	+
HAM–400	+	+
HAM–600	+	+
HAM–800	+	+

+ effective; − ineffective

3.4. TEM observations

Fig. 5 shows transmission electron microscope (TEM) photographs of Mont–400 and HAM before and after heating. There were no particles in Mont–400. In HAM–0, however, many fine spots are seen. Silver metal could not be observed in its X-ray diffraction profile. These spots may therefore represent certain material containing Ag, of which further details are not clear. With increasing temperature, such spots disappeared gradually and the large black spots (silver metal particle) appeared instead.

3.5. Antimicrobial tests

Table 1 shows results of antimicrobial tests. As shown

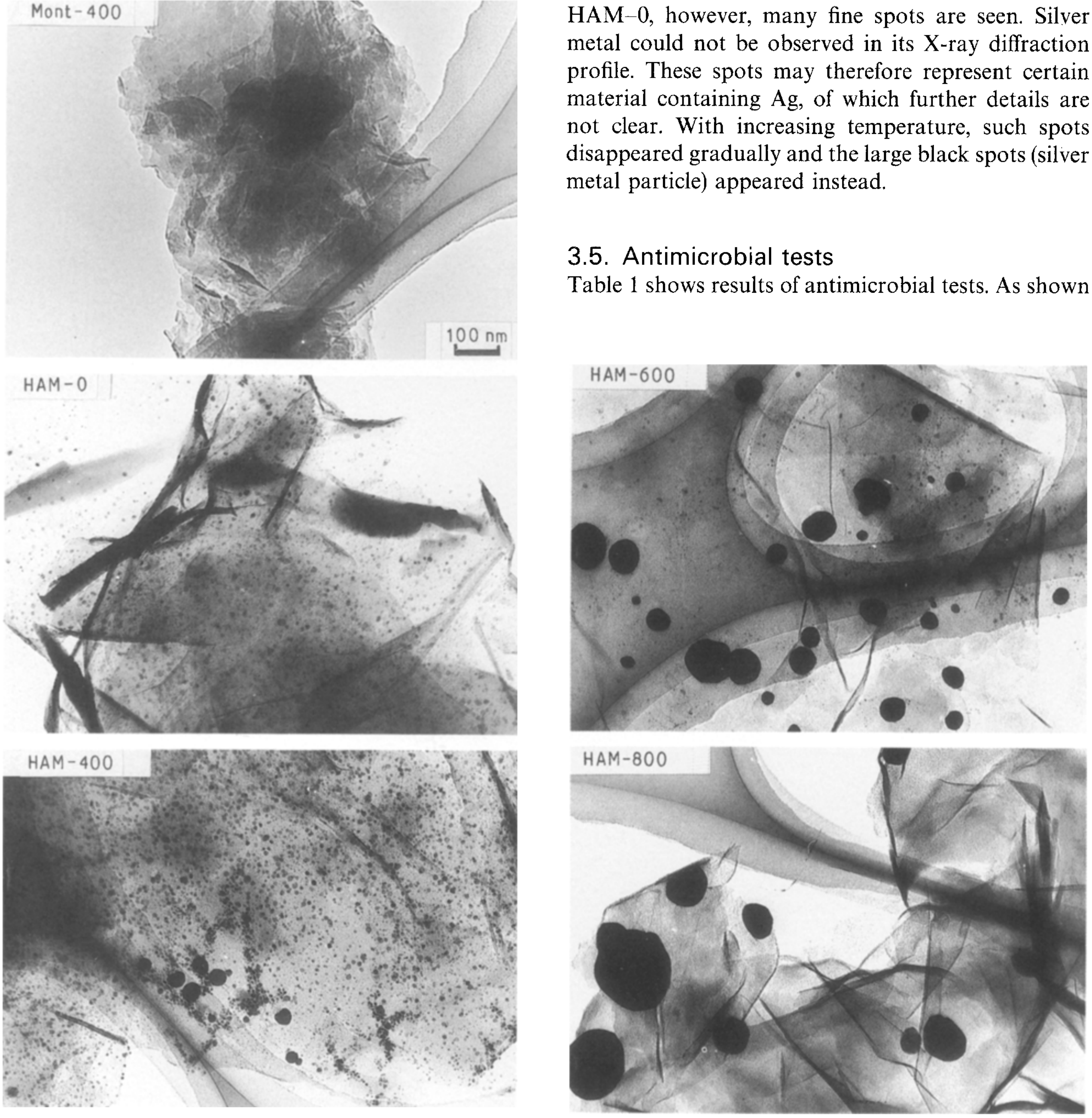

Figure 5 TEM photographs of Mont–400 and HAM before and after heating.

in this table, hypoxanthine has no antimicrobial activity. HAM showed clearly antimicrobial activity against both bacteria even after heating to high temperature. However, the width of the halo decreased with increasing temperature, indicating gradual decrease of antimicrobial activity, though not shown here.

4. Discussion

As reported previously [2], the reduction of the incorporated Ag^+ into metal was prevented by using Ag^+–chelates of 1,10-phenanthroline and 2,2′-bipyridine, without losing the antimicrobial activity of the agent. However, we could not make clear the role of the chelated Ag^+, because both chelating agents themselves showed antimicrobial activities. Hypoxanthine used here has no antimicrobial activity but its chelate of Ag^+ showed a clear activity as shown in Table I. It is concluded that Ag^+ possesses antimicrobial activity after chelating with hypoxanthine. Since the coordination number of hypoxanthine to Ag^+ is unity, Ag^+ is never covered completely by the hypoxanthine. It is quite reasonable that the chelated Ag^+ possesses antimicrobial activity.

Ag^+ in HAM is not reduced even after allowing to stand for a long time. A main role of a chelating agent is to prevent such reduction of Ag^+ into metal. A chelating agent seems to have additional roles as follows: (i) as shown in Fig. 1, Ag metal deposited in HAM after heating to 600 °C which is higher than the cases of Ag^+–chelates of 1,10-phenanthroline and 2,2′-bipyridine by 200 °C [2]. It is possible to control the deposition temperature of Ag metal particles by selecting a chelating agent. The deposition of Ag metal particles is known to lead to lowering of antimicrobial activity. In order to control the thermostability of this type of antimicrobial agent, the selection of the chelating agent is quite important; (ii) as reported previously [1], Ag–Mont was ineffective to certain moulds. The chelating agent in such Ag^+–chelates can show its ability in view of the chelate structure. It may be possible to prepare a unique agent possessing multi-functionalities from both Ag^+ and a chelating agent. For example, a strong antimicrobial and antifungal agent must be prepared by using Ag^+ and a chelating agent with a strong antifungal activity.

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References

1. A. OYA, T. BANSE, F. OHASHI and S. OTANI, *Appl. Clay Sci.* **6** (1991) 135–142.
2. F. OHASHI and A. OYA, *ibid.* **6** (1992) 301–309.
3. A. OYA, Y. OMATA and S. OTANI, *J. Mater. Sci.* **20** (1985) 255–260.

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